REMARKS/ARGUMENTS

In this preliminary amendment, claims 1-5 have been cancelled and claims 6-15 have been added. Accordingly, claims 6-15 remain pending in the present application.

Amendments to the Claims

Applicant has added new claims in this preliminary amendment. Applicant submits that the new claims are fully supported by the specification as filed and contain no new matter. For example, support for new claims 6-15 is found at page 6 and 12, of the specification as filed, which recites, in relevant part: *"TBO (e.g., prepared in accordance with Example 1 of my U.S. Patent No. 6,086,852)…"* and *"TBO (e.g., the product of Example 1 of U.S. Patent 6,086,852), raspberry flavoring agent (IFF raspberry IC563457), sodium acetate trihydrate buffering agent and H2O2 (30% USP) preservative (see U.S. Patent 5,372,801), are dissolved in purified water (USP), glacial acetic acid and SD 18 ethyl alcohol, to produce a TBO test solution, having the composition indicated in Table A…"* Applicant submits that the '852 Patent discloses the ratios of the different structures of toluidine blue (also referred to herein and in the'852 Patent as "peaks"), the percentage(s) by weight of certain structures in the composition, and the methods of preparing the same, as is claimed in the new claims of the present application. Please note that "peaks" refers to the 254nm HPLC peaks of toluidine blue. Accordingly, the compositions as claimed in the present invention incorporate the toluidine blue composition of the '852 Patent (and even Applicant submits

that TBO is prepared in accordance with the '852 Patent, as it was Applicant's intention to do so). Accordingly, please note that Applicant contemplated that the best mode of carrying out the present invention was use of the TBO composition as disclosed in the '852 Patent.

Additionally, Applicant has cancelled claims 1-5 herein in order to expedite prosecution of this application, and not to patentably define the subject matter over the prior art. Applicant expressively reserves the right to pursue the subject matter of the cancelled claims in a continuation application.

I. **35 U.S.C. Section 103(a)**

Claims 1-5 are rejected under 35 U.S.C. 103(a) in the parent case as being unpatentable over Mashberg ('251 Patent) et al in view of U.S. Patent No. 5,372,801 ('801 Patent) to Malmaro, and in further view of Rosin (see prior Office Action for the Rosin citation).

Specifically, Examiner recites in part on pages 4-5 of the Office Action:

Mashberg teach wherein the staining dye is toluidine blue. However, Mashberg do not expressly teach that the toluidine blue is toluidine blue O having the chemical abstracts service number (CAS) registry number 92-31-9. However, given the use of the toluidine blue, one of ordinary skill in the art would assume that the toluidine blue of Mashberg is the same as that of the instant invention. Nonetheless, Malmros teach a biological stain composition for in situ delineation of epithelial cancer wherein the stain composition comprises toluidine blue O having the CAS registry number 92-31-9. The chemical structure being phenothiazin-5ium, 3-amino-7-(dimethylamino)-2-methyl-, chloride.

Applicant respectfully disagrees and submits that the rejection is now moot since claims 1-5 are cancelled. Applicant submits that new independent claims 6, 7, 13, and 15 are patentable over Mashberg, Malmros, and Rosin. Claims that depend thereon are patentable in the same manner, as they contain all the limitations of the independent claims.

Mashberg discloses a TBO product that is not necessarily fit for human diagnostic use. In fact, Mashberg does not disclose the contents/composition of its "TBO product" whatsoever (only that the TBO product stains the nucleus). One skilled in the art cannot determine the identities isomers/N-demethylation derivatives of TBO and their ratios/respective percentages by weight in the composition. Accordingly, the '251 Patent discloses the term "toluidine blue O" ***without anything more***.

However, Malmros does not cure the deficiencies of Mashberg. Malmros discloses the "chromo" form of toluidine blue, ***without anything more***. Malmros does not disclose the relative amounts of the different species of toluidine blue in the "chromo" form of toluidine blue. How is one skilled in the art supposed to decipher the relative amounts of the different structures/peaks (conformational isomers and N-demethylation derivatives of toluidine blue) from the disclosure of Malmros, which discloses simply the "chromo" form? Malmros does not even recite the ***presence*** of different structures/peaks let alone their relative amounts in the dye composition. Is one skilled in the art supposed to simply "guess" that "chromo" means peak 8 and peak 7 in certain ratios or that the "chromo" form is formed by adding a complexing agent prior to the formation of the

third reaction mixture as is recited in independent claim 18?

As one skilled in the art can appreciate, "chromo" simply means "color." As such, Malmros simply discloses methods to prevent degradation of the toluidine blue composition due to it sitting on the shelf (i.e., by oxidizing the leuco form of the toluidine blue by adding an oxidizing agent). As one skilled in the art can appreciate from reading Malmros, reduction of toluidine blue causes reduction/loss of the ability of toluidine blue to stain. Malmaros simply discloses methods to prevent loss of this staining ability. Malmros apparently compares the staining ability of the leuco form of toluidine blue to the chromo form of toluidine blue and discloses that adding oxidizing agents maintains a "more" chromo state of the dye. Malmros teaches that the toluidine blue O stain solution should be freshly prepared and/or any leuco form present in the stain composition should be oxidized to the chromo form.

Preparation of the stain composition is accomplished by dissolving an effervescent tablet, containing an effective preselected quantity of the dye, in a preselected quantity of aqueous solvent. Conversion of the leuco form of the dye to the chromo form is accomplished by including in the stain composition a pharmaceutically acceptable oxidizing agent for leuco toluidine blue O. Accordingly, Malmros, as well as Mashberg, teach nothing of a pharmaceutically acceptable (pharmaceutical-grade) active ingredient, i.e., toluidine blue. Further, Malmros, as well as Mashberg ***do not*** disclose the relative amounts of the conformational isomers of toluidine blue to the amounts of

N-demethylated derivatives and/or their specific ratios as presently claimed.

Generally, in the prior art compositions (such as the prior art composition of the Mashberg and Malmros), the conformational isomers of TBO plus the N-demethylation and N,N-demethylation derivatives were less than 80% of the dye composition and the two N-demethylation derivatives formed greater than about 20% of the dye composition. Prior art workers were unaware of the exact composition of their "TBO" products and manufacturers of prior art TBO products were unable to reproducibly produce them. In fact, the prevalent literature description of the quality of TBO was simply "toluidine blue of good color value," i.e., toluidine blue in a *chromo* form as Malmros discloses. The prior art use of such loosely defined "TBO" resulted in differing clinical observations and problems obtaining the necessary regulatory clearances to manufacture and market such products for use in human diagnostic procedures.

On the other hand, independent claims 6, 7, 13, and 15 of the present invention recite in part, ***"the conformational isomers of toluidine blue O, the compounds having the structures [peak 8 and peak 7-see claim for the structure] ; (2) the N-demethylation derivatives of said isomers, the compounds having the structures [peak 6 and peak 5- see claim for the structure] the ratio of the combined areas of the 254 nm HPLC peaks representing said isomers to the combined areas of the peaks representing***

said N-demethylation derivatives being at least about 6:1…" or

"…the conformational isomers of toluidine blue O, the compounds having the structures [peak 8 and peak 7- see claim for the structure] in which [peak 8-see claim for the structure] comprises at least 58% of the total organic dye content of said composition." or

"…topically applying to epithelial tissue a dye that selectively stains cancerous and precancerous tissue…wherein the dye is made using a process for manufacturing toluidine blue O, which comprises the steps of…wherein the improved process comprises introducing said complexing reagent to a reaction mixture before the formation of said third reaction mixture, said complexing reagent being a compound that forms with said N,N-dimethyl-p-phenylenediamine said first intermediate and/or said second intermediate, a complex that provides steric hinderence to demthylation thereof…"

Accordingly, the present invention as claimed includes the identities and the amounts of the conformational isomers of toluidine blue O and the demethylation derivatives thereof relative to each other, and methods of diagnosing cancer comprising applying toluidine blue manufactured in part by the addition of a complexing agent ***prior*** to the formation of the third reaction mixture (as claimed in independent claim 13). For example, see FIG. 1

of the '852 Patent for the 254nm HPLC of the prior art TBO dye compositions, i.e., that of Mashberg or Malmros, and see FIG. 2 of the '852 Patent for the 254nm HPLC of the TBO composition as claimed in the methods of diagnosing cancer of the present invention. FIG. 1 is a 254nm HPLC chart depicting the peaks which were typically characteristic of TBO product compositions which were previously known (i.e., those disclosed, for example, in the '251 Patent). Note that in these prior TBO compositions, the combined area of peaks 7 and 8 was relatively low compared to the combined area of peaks 7 and 8 of the present invention. FIG. 2 is a 254nm HPLC chart depicting the peaks which are characteristic of TBO product compositions of the present invention. Note that in the TBO composition of the present invention, the combined area of peaks 7 and 8 is ***much higher*** than the combined areas of the peaks 7 and 8 of FIG. 1 (and, as claimed, peak 8 is at least 58% of the dye composition). For specific data, compare for example, the area of peak 8 in the prior art (i.e., the '251 Patent), 90.21, with the area of peak 8 in the present application, 270.765. We note that the greater the amount of peak 8 (one of the conformational isomers of toluidine blue) in the dye composition, the greater the purity of the stain.

Accordingly, the TBO content of the present invention has a higher content (i.e., percentage by weight) of peaks 7 and 8 because a complexing agent is added to the reaction mixture ***before*** the formation of the third reaction mixture as claimed in independent claim 13. Introducing the complexing agent prior to the formation of the third reaction mixture, i.e., prior to the oxidation of the indamine thiosulfonic acid and complexing the resultant TBO

reaction product to form the TBO-complex, produces a TBO-complex product having an improved ratio of TBO conformational isomers to the N-demethylation products thereof.

Please note that the greater the amount of peak 8 (the staining component of the dye), the greater the staining. The greater the staining, the more effective the dye is.

Additionally, on page 6 of the Office Action, the Examiner recites: "Regarding claims 3-5, Rosin et al teach wherein the microsatellite analysis is conducted at any one or a combination of chromosomes 3p and 9p and 17p..." In response, Applicant submits that since claims 3-5 are herein cancelled, the rejection is now moot.

Accordingly, present claims 6-15 distinguish over Mashberg, Malmros, and Rosin.

CONCLUSION

Accordingly, it is believed that all claims now pending patentably define the subject invention over the prior art of record and are in condition for allowance and such action is earnestly solicited at the earliest possible date. Please charge any underpayment, or credit any overpayment, to Deposit Account No. 10-0440.

Respectfully submitted,
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